

Will Allergen Fusion Bring New Energy to Immunotherapy?

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Although specific immunotherapy is currently only performed with aqueous allergen extracts, second-generation allergy vaccines may rather rely upon well-defined recombinant allergens [1]. The latter represent an attractive alternative to natural allergen extracts, since they can be produced in large quantities with consistent quality. In most circumstances, however, multiple allergens are involved in sensitization, making the recombinant allergen approach very complex as it requires a comprehensive characterization of each individual component of the vaccine. In this context, an interesting strategy is to fuse together several wild-type or mutated allergens, or peptidic fragments, using recombinant DNA technologies (fig. 1). In such fusions, allergens can be further linked to heterologous molecules, used as carriers to enhance stability and immunogenicity (e.g. by facilitating uptake by antigen-presenting cells), thereby creating hybrid or chimeric molecules. Such carrier molecules include, for example, viral proteins such as the rhinovirus or Q β phage capsid proteins, the pre-S protein from the hepatitis B virus, a modular antigen-translocating moiety, the Fc fragment of an immunoglobulin and the bacterial surface S-layer protein (fig. 1).

The fusion or hybrid approach has previously been applied by various groups to assemble head-to-tail allergens

from either grass or tree pollens, insect venoms, cat dander as well as house dust mites [2–13] (fig. 1). Such fusion proteins can then be expressed in *Escherichia coli*, yeasts (e.g. *Saccharomyces cerevisiae* or *Pichia pastoris*) and plant or mammalian host cell systems prior to purification and refolding. One advantage, obviously, is to produce the multiallergen drug substance as a single molecule, thus facilitating manufacturing and development. Another foreseen interest is to reduce IgE binding to the allergen(s), while enhancing its (their) capacity to elicit IgG and T cell responses [3, 10, 13].

In a recent issue of *International Archives of Allergy and Immunology*, Fujimura et al. [14] describe a fusion protein, involving Cry j 1 and Cry j 2 major allergens from Japanese cedar (JC) pollen, as a candidate product for specific immunotherapy. After only minor modifications, these 2 allergens were further conjugated with polyethylene glycol (PEG), commonly used in combination with therapeutic proteins to increase their stability in the bloodstream. JC pollen allergy is the most prevalent seasonal allergic rhinitis in Japan, and, as such, represents a major health problem with up to 25% of the population being sensitized. The PEG-fusion was expressed in *E. coli*, purified, and injected subcutaneously 4 times into Cry j 1-sensitized mice prior to a subcutaneous challenge with

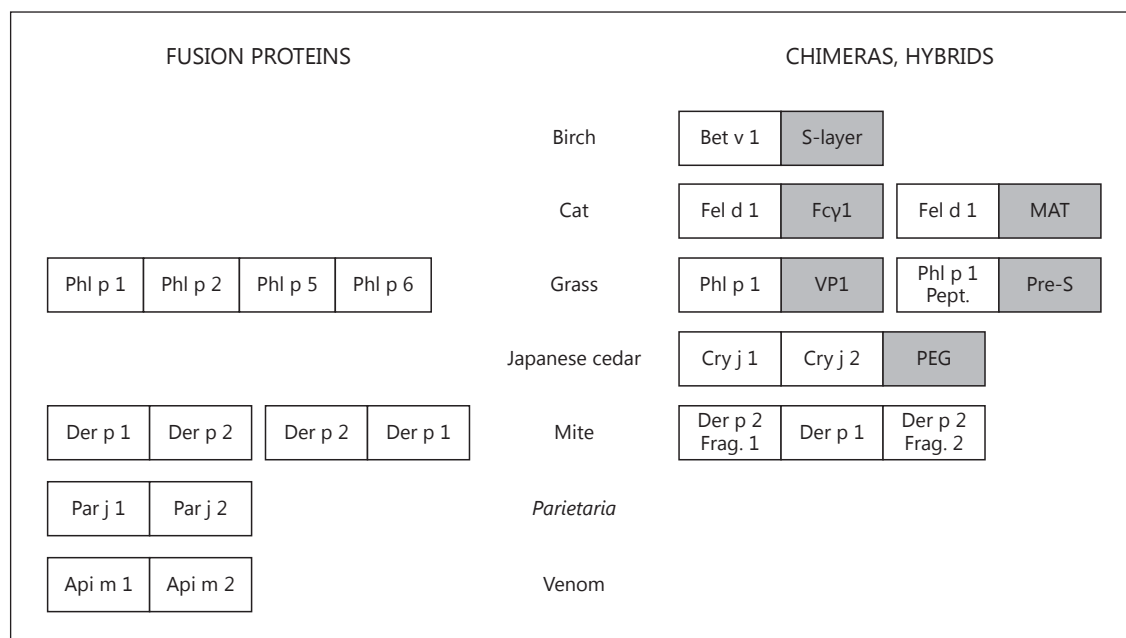


Fig. 1. Overview of fusion proteins and chimeras or hybrids made with allergens. In fusion proteins, allergens are assembled head-to-tail using recombinant DNA technologies. In chimeric and hybrid molecules, allergens or derived fragments (Frag.) or peptides (Pept.) are fused, with or without carrier molecules (gray boxes).

The latter encompass the viral proteins VP1 from rhinovirus, Qβ phage capsid protein and pre-S from hepatitis B, modular-antigen translocating (MAT) protein, the Fc fragment of IgG1 (Fcγ1), the bacterial surface S-layer protein or PEG.

Cry j 1. The PEG-fusion protein was also administered to monkeys who were subsequently challenged intranasally with the JC pollen extract. The induction of Cry j 1-specific IgEs was significantly attenuated in both the PEG-fusion-treated mice and the monkeys after challenge with Cry j 1 or the JC pollen extract, respectively. Proliferation and cytokine production by Cry j 1-specific Th2 cells were also reduced in the PEG-fusion-treated mice. Although regulatory T cells were not analyzed in these experiments, IFN-γ-secreting Th1 cells were increased in the PEG-fusion-treated groups. In parallel, Cry j 1-specific IgGs were significantly increased after PEG-fusion treatment in both the mice and the monkeys. On the basis of these promising results, the PEG-fusion can be considered an interesting candidate for the immunotherapy of patients allergic to JC pollen [14].

Importantly, in future animal models and in clinical studies, it will be interesting to compare the efficacy of the fusion protein with the JC pollen extracts that are currently available to treat patients. In addition, as suggested by the authors themselves, further work is needed to better understand the mechanisms at play when using the PEG-fusion, most particularly in relation to a potential

induction of JC-specific regulatory T cell responses. In this regard, since the fusion protein also encompasses the major allergen Cry j 2, it is expected that administration of the PEG-fusion would also downregulate Cry j 2-specific Th2 responses.

Although the fusion approach allows the simplification of product development by restricting the manufacture, purification and characterization steps to just a single ‘giant’ molecule, several potential drawbacks exist. First of all, in some studies, an inherent instability of fusion proteins has been observed in the form of degradation products or aggregates, e.g. when assembling Der p 1 and Der p 2 mite allergens [11, 12]. In this latter case, inappropriate cysteine pairing that created intermolecular disulfide bonds was involved as a cause of multimerization and aggregation [12]. Furthermore, disparate results have been obtained in expressing allergens with a proper conformation, depending upon the molecules fused. For example, a complete loss of secondary structure was observed for a *Parietaria judaica* Par j 1-Par j 2 fusion protein [7]. In contrast, a grass pollen chimeric protein assembling Phl p 1, Phl p 2, Phl p 5 and Phl p 6 appeared to be well refolded, exhibiting a circular dichro-

ism spectrum similar to the mixture of corresponding allergens [2]. Difficulty in completely refolding some fusion proteins might result from different folding characteristics for the various allergen components. Also, the head-to-tail fusion of allergens in the absence of a flexible linker inevitably causes structural constraints on each molecule, thereby impairing refolding. Although this can be considered a significant advantage with the aim of creating hypoallergens, it is a limitation of the fusion protein approach when a natural conformation is desired. Poor folding would make fusion proteins unsuitable for diag-

nostic purposes for which 'natural-like' recombinant molecules are obviously needed. Similarly, immunotherapy via the sublingual route requires allergens in a natural conformation to allow IgE-addressing to oral dendritic cells.

In this context, the use of a fusion protein approach is recommended for allergens that are easy to refold or, alternatively, for the development of hypoallergenic vaccines for which this approach will reduce IgE-binding while preserving immunogenicity in terms of IgG and T cell responses.

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